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AN 71:124896 CA
TI Synthesis and structure of fungisporin
AU Studer, Rolf O.
CS Chem. Res. Dep., F. Hoffmann-La Roche and Co. A.-G., Basel, Switz.

SO Experientia (1969), 25(9), 899
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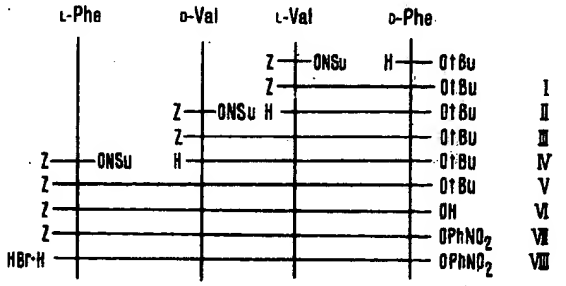
Synthesis and Structure of Fungisporin

In 1952 SUMIKI and MIYAO[1] reported on Fungisporin, a cyclooctapeptide isolated from spores of several species of *Penicillium* and *Aspergillus* as a crystalline sublimate by destructive distillation. Acid and alkaline hydrolysis yielded DL-valine and DL-phenylalanine in equimolar amounts. From the absence of terminal groups, the IR-spectrum, the solubility characteristics and the molecular weight of 980, determined by isothermic distillation in trifluoro-acetic acid, MIYAO[2,3] was able to propose the empirical formula cyclo-(Phe-Val)$_4$. Sequence studies and enzymatic experiments on peptide fragments, obtained by partial hydrolysis of fungisporin showed that the formula would most probably be cyclo-(D-Val-L-Val-D-Phe-L-Phe-)$_2$[3].

For the synthesis of this compound the tetrapeptide Z-L-Phe-D-Val-L-Val-D-Phe-OtBu V was prepared via the stepwise elongation method using the N-Hydroxysuccinimidesters of the corresponding Z-Aminoacids (reaction scheme).

Treatment of V with trifluoroacetic acid yielded the acid VI, which was transformed into the activated ester VII with di-(*p*-nitrophenyl) sulfite[5]. After the removal of the benzyloxycarbonyl group with HBr/acetic acid the resulting tetrapeptide *p*-nitrophenylester VIII was submitted to cyclization under high dilution in pyridin[6].



Reaction scheme[4]

From the residue obtained after evaporation of the solvent a crystalline, highly insoluble compound could be isolated by sublimation. The data obtained were in good agreement with the ones published for natural fungisporin with the exception of the molecular weight, which was found to be 482 by mass spectrometry. This is half the value found for natural fungisporin by the isothermic distillation method. A redetermination of the molecular weight of the natural compound[7] by mass spectrometry reveiled, however, also a molecular weight of 482. Fungisporin is therefore a cyclotetrapeptide and identical with cyclo-(L-Phe-D-Val-L-Val-D-Phe-).

Zusammenfassung. Die Struktur von Fungisporin, eines Zyklopeptides aus Sporen verschiedener Spezies von *Penicillium* und *Aspergillus*-Arten, wird durch Synthese und Vergleich mit dem Naturprodukt als die eines Zyklotetrapeptides, cyclo-(L-Phe-D-Val-L-Val-D-Phe-), bewiesen.

R. O. STUDER

Chemical Research Department,
F. Hoffmann-La Roche and Co. AG,
CH-4002 Basel (Switzerland), 18 June 1969.

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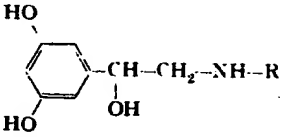
[2] K. MIYAO, Bull. agric. chem. Soc. Japan *19*, 86 (1955).
[3] K. MIYAO, Bull. agric. chem. Soc. Japan *24*, 23 (1960).
[4] Abbreviations: Amino-acids and peptides are abbreviated as recommended by the committee on Nomenclature which reported at the 5th European Peptide Symposium, Oxford, 1962, Proceeding (Ed. G. T. YOUNG, Pergamon Press, 1963). In addition: Z = benzyloxycarbonyl; OtBu = tertiary butylester; ONSu = N-hydroxysuccinimidester; OPhNO$_2$ = *p*-nitrophenylester.
[5] B. ISELIN and R. SCHWYZER, Helv. chim. Acta *43*, 1760 (1960).
[6] R. SCHWYZER and P. SIEBER, Helv. chim. Acta *40*, 624 (1957).
[7] We kindly thank Dr. K. MIYAO for supplying us with a sufficient amount of natural fungisporin for this comparison.

Two New Groups of Selective Stimulants of Adrenergic β-Receptors

Sympathomimetic agents acting on the adrenergic β-receptors are widely used in the treatment of bronchial asthma. Since both bronchodilatation and excitation of cardiac muscle are mediated by stimulation of the adrenergic β-receptors, bronchodilatation is often accompanied by tachycardia and palpitations. However, recent observations by LANDS et al.[1-3] indicate that the adrenergic β-receptors in the heart are different from those in the lung.

We have synthetized and tested pharmacologically a series of compounds of the following general formula:



where R is a branched alkyl group or cycloalkyl group, equal to *t*-butyl, *t*-pentyl, *t*-hexyl, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The compounds were tested for bronchospasmolytic effect in vitro on the isolated guinea-pig trachea and the effect on heart muscle on the right guinea-pig auricle (spontaneously beating). The effect on the tracheal muscles and on the heart muscle did not run parallel within the alkyl and the cycloalkyl series as can be seen from Figures 1 and 2. In the alkyl series maximal effect on the tracheal muscles was obtained for R = *t*-butyl, with an effect corresponding to 0.8 that of (−)-adrenaline. For R = *t*-pentyl and *t*-hexyl the effect on the trachea decreased. The effect on the right guinea-pig auricle was most pronounced for R = isopropyl (0.4 × (−)-adrenaline). With increasing size of the substituent, the effect on this preparation decreased.

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AU Cavelier-Frontin, Florine; Achmad, Sadijah; Verducci, Jean; Jacquier, Robert; Pepe, Gerard

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How to perform small peptide cyclizations

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Abstract

Small cyclopeptides of four to six residues are very interesting for their biological properties. Unfortunately, the synthesis of the linear precursor is generally fastidious and the cyclization often occurs in low yields. Molecular modeling used through the GENMOL program is a powerful tool for predicting the best precursor, as was shown in a previous paper about five tetrapeptides. However, sometimes all the linear precursors of a cyclopeptide can be unfavorable for cyclization when no structural feature (*N*-Me amino acid, Pro, D-amino acid) is present in the peptide. This led us to develop a method using a reversible chemical modification of the peptide main chain in order to favor the cisoid conformation able to cyclize easily. Tetraphenylalanine was used as a model, with the *tert*-butyloxycarbonyl (Boc) group as substituent on the main-chain nitrogen atoms. The cyclization yield increases from less than 1% to 27% after this chemical modification and cleavage of the Boc groups. Molecular modeling on such molecules shows that this yield increase is due to a preferred conformation having the terminal functions close together induced by the Boc substituents.

Introduction

Small cyclopeptides of four to six residues are of great interest because of their particular properties.

They are not recognized by exoproteases, so cyclic peptides are more resistant "in vivo" [1]. The crossing of lipidic membranes, leading to better bioavailability, is facilitated by the absence of charged extremities.

A cyclic peptide is often more active than a linear one, and sometimes corresponds to a more specific and more efficient drug.

Natural cyclopeptides or cyclodepsipeptides generally possess interesting biological properties: gramicidin [2,3] is an antibiotic; dolastatin 3 [4,5] is one of the most powerful antineoplastics known; peptides of the destruxin family [6,7] are very efficient insecticides; tentoxin [8–11] and HC-toxin [12,15] are phytotoxins; chlamydocin [16–20] exibits cytostatic and cancerostatic properties.

The various techniques suggested in the literature [21,22] for cyclization, which represents the limiting step, are often unsatisfactory.

Various factors are involved in the cyclization step. From a previous work [23] on five tetrapeptides (4-Ala-chlamydocin [24], HC-toxin [25], cyclotetrapeptides of sarcosine in combination with glycine [26], and 4-Ala-chlamydocin [27] and Cyl-2 [28] analogs) we found from modeling calculations performed with the GENMOL program [29] that the barrier to reach the transition state energy is the limiting factor in the cyclization reaction for these peptides.

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The rigidity of the linear precursor increases the difficulty of cyclization. Peptide bonds possess strong π character and preferentially adopt a transoid conformation [30]. The linear precursor is elongated with the terminal acid and amine functions in remote positions, this being unfavorable to intramolecular coupling. The presence of N-substituted amino acids [31] (imino acids) or D and L amino acids in alternate positions [15] re-establishes the transoid–cisoid equilibrium. The cisoid form is favorable for cyclization, as was suggested by Bhatnagar [32] from the tentoxin example. An experimental result concerning the cyclization of tetraglycine [26] and tetrasarcosine [33] under the same conditions confirms this effect. The yields of cyclomonomer are 5% and 43% respectively. If no natural factor favorable to cyclization exists, we can use a reversible N-substitution in order to shift the cisoid–transoid equilibrium to the cisoid conformer. In order to study the chemical substitution on the nitrogen atoms we chose tetraphenylalanine as a model.

This choice was made for two reasons:

There is only one closing site, which avoids the variations of the yield due to different possible precursors;

The cyclization yield is very low (< 1%) so any increase of the cyclization yield will be observed.

Tetrapeptide cyclizations

4-Ala-chlamydocin

In a previous work [23] calculations performed on the transition state of the cyclization reaction of the four precursors of 4-Ala-chlamydocin: (**1**, Phe–D-Pro–Ala–Aib–OΦ; **2**, D-Pro–Ala–Aib–Phe–OΦ; **3**, Ala–Aib–Phe–D-Pro–OΦ; **4**, Aib–Phe–D-Pro–Ala–OΦ) indicate clearly that the barrier (activation energy) in order to reach this state was the main factor in the cyclization of these peptides (if compared to the other factors like dimerization, for example). The transition state displayed in Fig. 1 is modeled based on the association N···C=O observed in the crystal state [34] of other nonpeptide compounds. The energy height is considered as the difference between the strained geometry, in which distances are imposed as indicated in Fig. 1, and the relaxed geometry, which is the closest to this strained state.

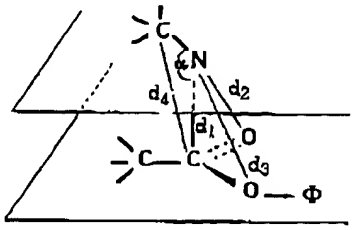

Fig. 1. Geometry of the transition state under the constraints imposed in the calculations.

The calculations were performed on the charged forms chosen in order to illustrate the nucleophilic and electrophilic partial charges of the transition state. The results corresponding to the geometries and the energies of the transition state before and after relaxation are reported on Table 1. Precursor **3** corresponds to the lowest energy difference (14 kcal mol^{-1}) compared to 55, 54 and 54 kcal mol^{-1} for precursors **1, 2** and **4** respectively.

The transition state energy for precursor **3** is also the lowest (61.7 kcal mol^{-1}) compared to 129, 106 and 103 kcal mol^{-1} for precursors **1, 2** and **4** respectively. These modeling results correspond to experimental observations [27]. In our previous work [23], the calculation results indicate that the dimerization reaction and the steric hindrance to ring closure are of secondary importance for cyclization. In order to check the general validity of the model, the study was extended to five other tetrapeptides: two HC-toxin analogs; cyclotetrapeptides of sarcosine with glycine; 4-Ala-chlamydocin; Cyl-2 analogs. In all cases, the best precursor is suggested by the modeling calculations.

Tetraphenylalanine

Chemistry

The steric effect of the N-substitution decreases

Table 1
Energies E (kcal mol^{-1}) and geometric parameters d, α (distances in ångströms, angle, in degrees) for the transition states of the 4-Ala-chlamydocin precursors in strained and relaxed forms

Form[a]	Parameter	Precursor[b]			
		1	2	3	4
A	E	129	106	61.7	103
	d_1	2.77	2.71	2.76	2.78
	d_2	3.05	3.07	3.08	3.03
	d_3	3.14	3.12	3.13	3.12
	d_4	3.24	3.23	3.22	3.25
	α	94.5	96.3	94.7	94.5
B	E	78.4	52.7	46.9	51.4
	d_1	3.23	2.95	2.93	3.03
	d_2	3.22	3.70	3.22	3.03
	d_3	3.71	3.14	3.33	3.44
	d_4	3.60	3.70	3.57	3.95
	α	91.6	108.3	102	116.2
C	E	73.7	52.0	47.6	48.8
	d_1	3.06	2.94	2.97	2.98
	d_2	3.13	3.74	3.15	3.22
	d_3	3.31	3.04	3.46	3.50
	d_4	3.98	3.79	3.66	4.12
	α	117	113.7	105	134
ΔE(A–C)		55	54	14	54
Cyclization yield (%)		2	3	45	3

[a] A, strained state; B, relaxed by force field calculations; C, completely relaxed after rotation around pivots [23].
[b] Precursor 3 is the most favorable for cyclization (14 kcal mol^{-1} is the lowest energy to reach the transition state). 1, Phe–D-Pro–Ala–Aib–OΦ; 2, D-Pro–Ala–Aib–Phe–OΦ; 3, Ala–Aib–Phe–D-Pro–OΦ; 4, Aib–Phe–D-Pro–Ala–OΦ.

the π character of the peptide bond, which facilitates rotation around this bond and thus re-establishes the equilibrium between the transoid and cisoid populations. The rotation barrier is estimated to be around 20 kcal mol^{-1} [31], which is confirmed by calculations with the GENMOL program: the energy decreases from 22.6 to 10.5 kcal mol^{-1} if a Boc group is added on the nitrogen atom, a result which confirms the increase of the cisoid conformer population when the nitrogen atom is substituted.

This substitution effect led us to develop a method of reversible chemical modification of the peptide main-chain conformation. The best result was obtained with the Boc substituent; an experimental procedure used to add the Boc group on the nitrogen atom is described by Gunnarsson et al. [35,36]. In Fig. 2 the main steps of the synthetic route to prepare the "over Boc" Phe_4 are displayed. This modification of the main-chain improves the yield of cyclization from less than 1% to 27%. Other attempts using different substituents have failed to prepare linear precursors [37].

Modeling of the tetraphenylalanine cyclization

Molecular modeling was used in order to explain the significant increase of the experimental cyclization yield when Boc groups are added on the nitrogen atoms of the linear precursor.

In contrast to the 4-Ala-chlamydocin case previously studied, only one closing site exists for Phe_4 and its "over Boc" derivative. However, the same idea — that the transition state energy is the determining factor for the ring closure — directs us in the modeling approach. The geometry of the transition state was blocked and the corresponding energy was calculated, first under strain and then after relaxation.

The extremities of each linear peptide are progressively moved farther away (with a step of 1 Å) and at each step the molecule is relaxed. The starting point corresponds to a distance of 2.8 Å (the shortest distance without atom interpenetration). The results are displayed in Table 2 for Phe_4–O Act and in Table 3 for the "over Boc" Phe_4–O Act.

Some meaningful information can be drawn from this analysis, as follows.

Phe_4–O Act. The energy remains almost the same (Table 2) whatever the distances between the extremities 91.6 kcal mol^{-1} for 3 Å and 89.2 kcal mol^{-1} for 8 Å. When the molecule is relaxed at each calculation step, the energy well as the geometry remains equivalent to those of the strained state. These results indicate that there is no privileged geometry in the closing region for this molecule.

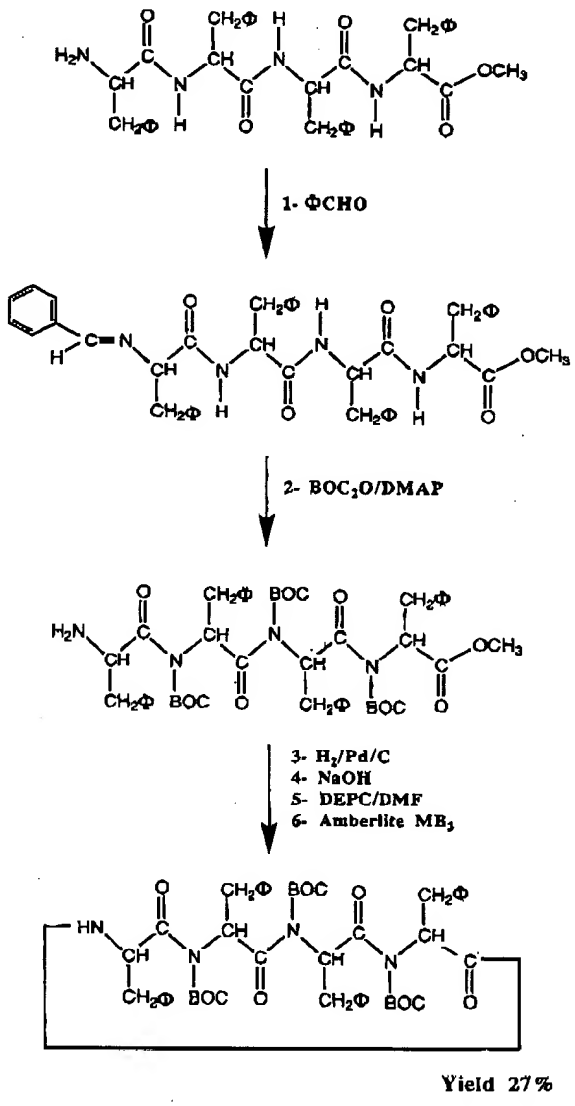

Fig. 2. The principal steps of the chemical method developed to obtain a cyclopeptide. Example corresponding to the cyclotetraphenylalaline: 1, protection of amine terminal function; 2, selective substitution of amidic nitrogen atoms; 3, regeneration of terminal amine function; 4, regeneration of terminal acid function; 5, cyclization; 6, Boc elimination and purification.

"Over Boc" Phe_4–OAct. The strain energy for this molecule is lower than in the Phe_4–O Act molecule, although a greater number of atoms is involved. The energy in the transition state is 35.5 kcal mol^{-1} instead of 94.3 kcal mol^{-1} for Phe_4–O Act, which means that the Boc substituents give more softness to the molecule, as is shown by the decrease of the rotation barrier around the peptide bond. The strain energy increases with the distance between the extremities: 70.1 kcal mol^{-1} for a distance of 8 Å, which is confirmed when the molecule is relaxed, the strain energy and the distance between the extremities decreasing systematically. For example, on the last point calculated (8 Å), the energy becomes 48.2 kcal mol^{-1} while the distance between the extremities becomes 6.02 Å after relaxation. These results show a small decrease in the energy barrier (from 3.5 to 0.8 kcal mol^{-1}) to reach the transition state, which is very low in both molecules and not the significant factor. However, these results also indicate that substitution of main-chain nitrogen atoms increases the softness of the molecule and stabilizes that conformation with both terminal functions close together, which explains the best experimental cyclization yield.

Conclusion

From molecular modeling with the program GENMOL it is possible to predict the best plausible precursor for cyclization, as was done on 4-Ala-chlamydocin and other small peptides [23] from the transition state energy analysis.

Sometimes all the precursors of cyclopeptide can be linear and rigid (unfavorable for cyclization), which led us to develop a method of reversible chemical modification of the peptide main-chain conformation, favoring the cisoid form. In all cases molecular modeling appears as a powerful tool to understand the experimental results and even allows them to be predicted, which saves much time and avoids useless synthesis experiments.

The method was defined using tetraphenylalaline as a model. In this case, the non-cyclization of this peptide is not related

Table 2
Energies E (kcal mol^{-1}) and geometric parameters d, α (distances in ångströms, angles in degrees) for Phe_4–O Act in the strained state and the relaxed state corresponding to the energy pathway for the ring closure[a]

Strained A	d_1	2.80	3.00	4.00	5.00	6.00	7.00	8.00
	d_2	3.05	3.07	3.39	4.22	5.52	6.85	8.06
	d_3	3.12	3.20	4.48	5.60	6.71	7.68	8.59
	d_4	3.17	3.68	4.24	4.93	5.53	6.17	6.87
	α	90	103	89	79	65	51	37
	E	94.3	91.6	89.8	89.0	88.2	88.1	89.2
Relaxed C	d_1	3.60	3.77	4.09	4.78	5.53	6.40	7.37
	d_2	3.40	3.49	3.36	3.96	4.76	5.71	6.83
	d_3	3.65	4.89	4.64	5.42	6.43	7.44	8.39
	d_4	3.89	4.14	4.44	5.61	5.16	5.61	6.34
	α	90	94	91	82	68	52	41
	E	90.8	90.6	88.1	87.7	87.3	87.4	87.0

[a] See entries A and C of Table 1. The molecule prefers to adopt a linear conformation (transoid) unfavorable for cyclization.

Table 3
Energies E (kcal mol^{-1}) and geometric parameters d, α (distances in ångströms, angles in degrees) for the "over Boc" Phe_4–O Act in the strained state and in the relaxed state corresponding to the energy pathway for the ring closure[a]

Strained A	d_1	2.80	3.00	4.00	5.00	6.00	7.00	8.00
	d_2	3.05	3.12	4.44	4.76	6.53	7.42	8.77
	d_3	3.12	3.03	3.52	3.94	4.82	5.65	6.73
	d_4	3.17	3.59	4.01	4.51	5.15	5.90	6.75
	α	91	100	80	64	52	43	31
	E	35.5	34.3	36.8	45.6	50.4	61.2	70.1
Relaxed C	d_1	3.24	3.40	3.64	4.01	4.72	5.41	6.02
	d_2	3.43	3.37	4.05	4.12	5.31	5.98	6.77
	d_3	3.09	3.19	3.14	3.38	3.72	3.33	4.78
	d_4	3.68	3.70	3.79	3.99	4.40	4.89	5.33
	α	96	90	92	82	69	61	55
	E	34.7	36.0	36.2	37.8	39.7	43.6	48.2

[a] See entries A and C of Table 1. The molecule prefers to adopt a closed conformation (cisoid) favorable for cyclization.

to the transition state energy, as it was for 4-Ala-chlamydocin and the other tetrapeptides previously studied, but to a conformational problem, i.e. the preferred linear and rigid conformation of this molecule is unfavorable for cyclization. However, addition of Boc groups on the peptide main-chain nitrogen atoms softens the molecule and induces a conformation with the extremities close together and able to cyclize.

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TI Synthesis of biologically active cyclic peptides and depsipeptides by the phosphite method
AU Rothe, M.; Kreiss, W.
CS Org.-Chem. Inst., Univ. Mainz, Mainz, Ger.

SO Pept., Proc. Eur. Pept. Symp., 14th (1976), 71-8.
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SYNTHESIS OF BIOLOGICALLY ACTIVE CYCLIC PEPTIDES AND DEPSIPEPTIDES BY THE PHOSPHITE METHOD

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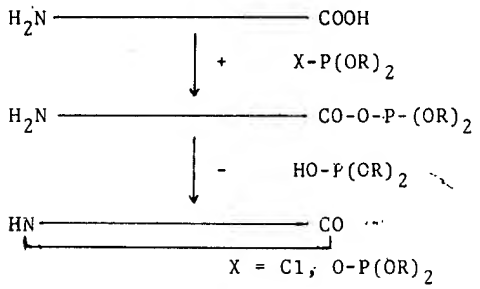
Despite the progress in the field of peptide synthesis during the last two decades, the cyclization of linear peptides still poses problems, particularly with respect to the yields obtained and the ring sizes formed. Although many active peptide derivatives and coupling reagents have been employed for cyclization, only moderate yields (up to 40%) are generally obtained. Yields are sufficient using the active ester method; with the azide method, recently preferred [1], difficulties may arise as a result of the formation of side products (amides, ureas) which may not be detected and which may be inseparable from the cyclopeptides because of their similar properties. Moreover, the absence of homologues with double or triple the ring size, has seldom been proved and, in most cases, not even investigated. The need for such investigation must be emphasized as we lately found ring closure even in very dilute solution leads to a whole series of homologous cyclic peptides of differing ring sizes.

The phosphite method has been used by us successfully in cyclization of simple model peptides [2], and it appeared of utility in the synthesis of lipophilic cyclic peptides and depsipeptides being of importance as antibiotics, ion carriers, and antitoxins.

Scheme 1: Cyclization of Peptides by the Phosphite Method



In the phosphite method unprotected linear peptides react with chlorophosphites and pyrophosphites to form mixed anhydrides which then yield cyclic products spontaneously by interaction with the free amino group at the other end of the chain. The advantages of the method lie in the use of strictly defined and stable starting materials (in contrast to the salts of active peptide esters and azides), the very simple handling techniques necessary and the efficiency of the ring closure (yields are usually higher than by any other method). Our preferred conditions involve the use of diethyl phosphite as solvent (most free peptides are readily soluble), a nitrogen atmosphere to avoid discolouration, not too low a concentration of the peptide (0.01 M), a short reaction time (30 min.), and a relatively high temperature (100°). Too high a temperature and extended reaction times (e.g. 10 hrs. at 140°), or the use of pyridine as solvent, lead to low or nil yields [3].

Too high a dilution must also be avoided because the activation of the peptide by chlorophosphite is bimolecular [4] and thus strongly concentration dependent. As active phosphite derivatives we have employed diethyl chlorophosphite, ethylene chlorophosphite and diethyl ethylene pyrophosphite, and others. However, we prefer o-phenylene chlorophosphite because it is easily accessible and stable.

Under these conditions, monomeric and oligomeric cyclic peptides and linear polypeptides are formed depending on the concentration and the chain length of the starting peptide. After removal of the linear peptides with macroporous ion exchangers, the resulting mixture of cyclic peptides is best separated by gel permeation chromatography. Individual rings can then be identified by mass spectrometry or vapour pressure osmometry.

Table 1: Synthesis of Biologically Active Cyclopeptides (Phosphite Method)

		Yield(%)
Gramicidin S (+ Semi-Gramicidin S)	c-(Val-Orn(Pht)-Leu-D-Phe-Pro)$_{1+2}$	87
Fungisporin	c-(D-Phe-Phe-D-Val-Val)	46
Antamanide	c-(Phe-Phe-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro)	59
Valinomycin	c-(Val-D-Hyv-D-Val-Lac)$_3$	56

We adopted the method for the synthesis of biologically active cyclopeptides and cyclodepsipeptides, e.g. gramicidin S and semi-gramicidin S [5], fungisporin [6], antamanide, valinomycin and their analogues. The yields of GPC-purified products range from 50 to 85%, i.e. they are considerably higher than the best reported in the literature.

We investigated the synthesis of valinomycin in detail [7]. Because of the symmetrical structure of valinomycin (which consists of three identical tetradepsipeptide units), cyclizations were carried out with the complete dodecadepsipeptide and also with the homologous tetra- and octa-depsipeptide partial sequences. As the ring contains alternating peptide and ester bonds, there are two possible starting sequences leading to peptide bonds. In accordance with Gisin et al. [8] and Losse et al. [9] we chose the depsipeptides with C-terminal lactic acid which were expected to be less sterically hindered than the alternatives.

After the cyclization reactions we obtained, besides valinomycin, a series of cyclic oligomers including the hitherto unknown 12-membered cyclo-tetradepsipeptide. Starting with the octadepsipeptide we obtained the expected 16-membered "octa"-valinomycin, a hexadecadepsipeptide formed by cyclodimerization, and a 72-membered tetracosadepsipeptide (the dimer of valinomycin). The volatility and stability of all the products permitted their identification by mass spectrometry up to molecular weights of more than 2000. "Tetracosa"-valinomycin, also prepared for the first time, conteins the largest ring size so far obtained in a cyclic depsipeptide. Its influence on cation transport through lipid membranes will be of interest. The 12-membered "tetra"-valinomycin showed surprising properties in comparison with its higher ring homologues. Whereas the larger rings were soluble even in hydrocarbons, no satisfactory solvent could be found for the cyclotetradepsipeptide. The high m.p. (285°) indicates a very stable crystal lattice. The IR spectrum gave no indication of cis-peptide bonds and strongly resembled that of valinomycin and the other larger rings; in particular, it contained a strong amide II band at 1560 cm^{-1}, an amide III band at 1290 cm^{-1}, and a NH stretching vibration at 3290 cm^{-1} as a proof of trans-peptide bonds. Strain-free molecular models of this 12-membered ring could be built with trans-peptide and trans-ester bonds.

We next examined differing conditions for the cyclizations and obtained information concerning ease of cyclization, formation of cyclic oligomers, influence of solvent polarity and concentration dependence. Cyclization of the linear tetra-, octa- and dodeca-depsipeptides, under the same conditions showed the dependence of ease of cyclization on ring size. Yields were 52, 74 and 56 % respectively for the 12-membered tetradepsipeptide, the 24-membered octadepsipeptide, and the 36-membered valinomycin. This indicates that the cyclic octadepsipeptide has a particularly high conformational stability.

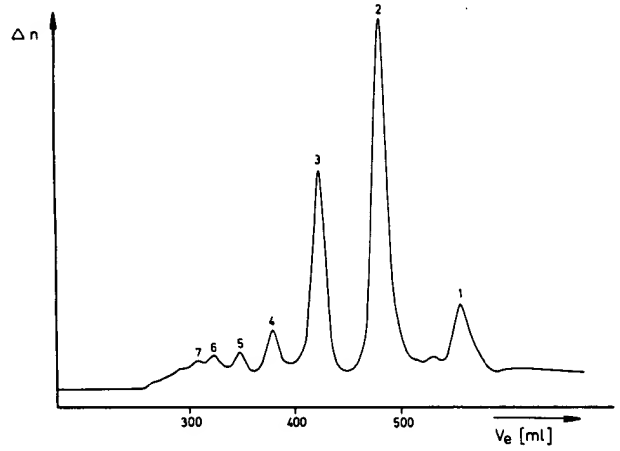


Figure 1: Valinomycin Oligomers, $(Val\text{-}D\text{-}Hyv\text{-}D\text{-}Val\text{-}Lac)_n$, gel permeation chromatography, Sephadex LH 20, methanol

The highest degree of cyclo-oligomerization was found with the tetradepsipeptide (Fig. 1). In this case all rings of the homologous series up to the heptacosapeptide (7 units, 84 ring atoms) could be detected and separated by GPC on Sephadex LH 20. As already mentioned, the formation of higher ring of oligomers was also observed with the octadepsipeptide, whereas the dodecadepsipeptide gave only valinomycin without detectable amounts of higher rings. The influence of the concentration of the linear peptide precursors on yields and ratios of the cyclic oligomers correspond to the Ziegler dilution principle. Thus, valinomycin could be obtained from the linear tetradepsipeptide (by cyclotrimerization) at higher concentrations only.

Table 2: Solvent Effect on the Cyclization of Valinomycin Sequences

$H\text{-}(Val\text{-}D\text{-}Hyv\text{-}D\text{-}Val\text{-}Lac)_n\text{-}OH$			$cyclo\text{-}(Val\text{-}D\text{-}Hyv\text{-}D\text{-}Val\text{-}Lac)_n$				
n	Solvent*	c (M)	yield %				
			n = 1	2	3	4	6
1	DEP	0.05	48	14	2	-	-
	TOL	0.05	4	13	7	2	-
2	DEP	0.01	-	30	-	4	0.7
	TOL	0.01	-	23	-	10	2
3	DEP	0.001	-	-	24	-	-
	TOL	0.001	-	-	56	-	-

* DEP = diethyl phosphite
TOL = toluene

Because of the high lipophilicity of the linear and cyclic depsipeptides we were able to examine nonpolar solvents for the cyclization. When changing from polar (diethyl phosphite) to nonpolar (toluene) solvents we observed clear differences in the ratios of ring oligomers obtained. Larger rings, rather than monomeric cyclodepsipeptides, resulted when toluene was used as solvent (Table 2). There may be several reasons for this effect: for example association of linear depsipeptides in nonpolar solvents and stronger solvation which imparts stiffness to the chains (causing thereby steric hindrance in the intramolecular reaction). The occurrence of association in toluene could be proved in the case of the linear tetra- and octa-depsipeptides of valinomycin by vapour pressure osmometry. But no relation between this association and the formation of larger rings could be found. For example a uniform increase in the amount of dimeric cyclohexadepsipeptide from octapeptide was found with increasing concentration, whereas vapour pressure osmometry indicated an abrupt change in the degree of association of the peptide chain in this range of concentration.

The formation of various ring homologues is therefore considered to be the result of a polycondensation, cyclization being a reaction competitive to chain propagation.

Table 3: Synthesis of Antamanide

Sequence A: Phe-Phe-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro (V_e = 363 ml)
Sequence B: Pro-Ala-Phe-Phe-Pro-Pro-Phe-Phe-Val-Pro (V_e = 428 ml)

Sequence	Method	c (M)	Solvent	Yield
A	Phosphite	0.01	DEP	32
B				17
A	Phosphite	0.001	DEP	42
B				18
A	Phosphite	0.001	C_6H_5Cl	59
B				45
A	DCC	0.003	CH_2Cl_2/DMF	50
B				1.5

An extraordinary influence of conformation of linear peptides on the cyclization reaction was found in the synthesis of antamanide. We studied its formation by the phosphite method starting with the linear decapeptides A and B, which differ only in the sequence of the amino-acids. Diethyl phosphite and chlorobenzene were used as solvents. Sequence A always gave considerably higher yields than sequence B, irrespective of the reaction conditions and the method of ring closure. The effect was particularly high when the carbodiimide procedure was

employed. Such striking differences cannot be attributed to differing rates of formation of the Pro-Phe or Pro-Pro bonds during ring closure. On the other hand, considerable deviations in the conformations of the two linear decapeptides were inferred from their behaviour in gel permeation chromatography. Full concordance of the elution volumes was expected because of their identical amino-acid compositions and molecular weights. But to our surprise we found large differences, i.e. 363 ml for sequence A and 428 ml for sequence B. Proof of the existence of very different conformations resulted from their very different CD spectra.

The advantages of the phosphite method over other methods are obvious. The DCC method has already been used by König and Geiger [10] for the cyclization of the sequence with C-terminal alanine (yield 36.5%). Sequence A was also cyclized by Wieland and coworkers [11]. In their work the nitrophenyl ester method yielded 29% of the cyclodecapeptide, whilst the anhydride procedure gave only 7%. By the phosphite method we obtained nearly 60% of antamanide.

Table 4: Synthesis of Valinomycin by different Methods

Method	Solvent	Yield (%)
Phosphite	Toluene	56
DCC	Toluene	31
DCC	Cyclohexane	21
Acid chloride	Benzene	41

Similar results were obtained in the ring closure of depsipeptide sequences (Table 4). In the cyclization of the linear dodecadepsipeptide precursor of valinomycin, the DCC, the acid chloride, and phosphite methods were examined. The phosphite method again proved superior with respect to the yields and purity of the cyclization products. This was evident after gel permeation chromatography of the reaction products. The acid chloride method yielded only coloured substances, whereas the phosphite method - using the same working up procedure - gave pure valinomycin directly.

In conclusion, the phosphite method can now be regarded as an advantageous alternative to other methods for the synthesis of biologically active cyclopeptides.

ACKNOWLEDGEMENT

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DISCUSSION

BRENNER — Can the amount of phosphite applied have accounted for a cyclization which is bimolecular with respect to phosphorus ?
This could give an explanation for the superiority of phosphite over the other reagents used in your work.

ROTHE — We always use a 4 to 6-fold excess of the chlorophosphite in order to favour anhydride formation in the dilute solution by mass action.

BIRR — Have you found cyclic oligomers of antamanide in your phosphite procedure ?
How do you analyze those oligomers?

ROTHE — We have only found a small peak in the gel chromatogram which corresponds to the expected elution volume for the dimer of antamanide.

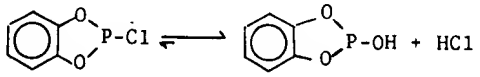
GROSS — How do the ionophoric properties of high molecular weight cyclopolymers of Valinomycin differ from those of native Valinomycin ?

ROTHE — We have sent some samples to Dr. Läuger who will carry out these studies.

UGI — Corey has recently reported that the Mukaiyama's method is particularly favourable in cyclization reactions.
Have you compared the phosphite method with the Mukaiyama's one ?

ROTHE — No, we have not.

RYDON — I think that the high acidity you have observed may be assigned to the reaction :



This is why we preferred the pyrophosphite in our original work many years ago.

ROTHE — The cyclization mixtures become strongly acidic even if you use a high excess of tertiary amine. The acidity may be attributed to a hydrolysis of the o-phenylene chlorophosphite to the monophosphite or to phosphorous acid.

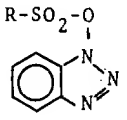
B.O.P.: A NEW PEPTIDE COUPLING REAGENT EXEMPLIFIED IN THE SYNTHESIS OF SOMATOSTATIN

B. CASTRO, J.R. DORMOY, G. EVIN and C. SELVE

(Laboratoire de Chimie Organique 2, E.R.A. 558 C.N.R.S., Nancy France)

1. INTRODUCTION

Various activated derivatives of N-hydroxybenzotriazole (HOBt) have been proposed recently as peptide coupling reagents; the sulphonates 1 have been described by Itoh [1], and our own group, following an investigation of the acyloxyphosphonium intermediates [2], suggested the Benzotriazolyloxy(trisdiméthylamino) Phosphonium hexafluorophosphate 2 the so called B.O.P. reagent) [3].



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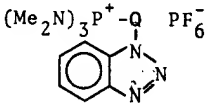

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We describe now a novel, cheaper preparation of the BOP reagent, the mechanism of active ester formation and a synthesis of the linear tetradecapeptide of somatostatin, which permits an evaluation of the reagent in fragment condensation.

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TI Cyclic peptide analogs of gastrin
AU Tritsch, G. L.; Sachatello, C. R.; Grahl-Nielsen, O.; Moriarty, C. L.; Sedwick, J.

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SO J. Med. (Basel) (1971), 2(2), 82-5
CODEN: JNMDBO

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J. Med. 2: 82–85 (1971)

Cyclic Peptide Analogs of Gastrin

G. L. TRITSCH, C. R. SACHATELLO, O. GRAHL-NIELSEN, C. L. MORIARTY and J. SEDWICK

General Clinical Research Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, N.Y.

Abstract. Cyclic analogs of two biologically active gastrin peptide analogs were synthesized to determine the effect of such a limited restriction on the many conformations these small peptides can assume in solution. Tryptophylmethionylaspartylphenylalanyl and Tryptophylmethionylaspartylphenylalanyltryptophylmethionylaspartylphenylalanyl were devoid of secretagogue activity and were unable to inhibit the activity of an active secretagogue. It is concluded that the gastrin receptors require not only the proper amino acid sequence but also a particular three-dimensional conformation of biologically active analogs of gastrin.

Key Words
Gastrin analogs
Cyclic peptides
Gastric secretagogues

The full range of the biological activity of gastrin is displayed by the carboxy-terminal tetrapeptide amide amino acid sequence of the hormone, Tryptophylmethionylaspartylphenylalanine amide, and acylation of the α-amino group of tryptophan may enhance potency [MORLEY, 1968]. Since removal of the amide from the phenylalanine residue abolishes the biological activity, it appears that neither end of the tetrapeptide should be free for optimum secretagogue activity.

Although several hundred analogs of this tetrapeptide amino acid sequence have been prepared [MORLEY, 1968], none of the compounds were designed to test specifically the effect of the three-dimensional folding of this small peptide chain on the secretagogue activity. Membrane diffusion studies have shown [CRAIG *et al.*, 1964] that the conformation of small peptides is sensitive to the ionic environment. Furthermore, secretin, which inhibits the acid secretagogue activity of pentagastrin in man [BROOKS and GROSSMAN, 1970] has part of its peptide chain stabilized as a cyclic structure [BODANSKY *et al.*, 1969]. To test if three-dimensional folding

has a part to play in determining biological activity of the short gastrin tetrapeptide analogs, we have synthesized cyclic peptide analogs with the intent of determining the effect of the imposition of this limited restriction on the many shapes the peptides can assume in solution.

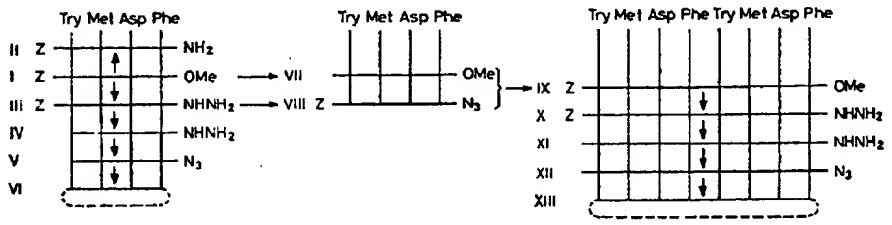

Fig. 1

Figure 1 outlines the synthesis of two cyclic peptides (VI and XIII) which are analogous to biologically active linear peptides. The starting material, compound I, was synthesized as described by DAVEY *et al.* [1966]. Removal of carbobenzoxy groups, abbreviated at Z in figure 1, was accomplished by hydrogenation at room temperature; azide coupling to produce cyclic peptides took place at 0°C for 7 days with the peptide concentration at 1 mM. At this low concentration, polymerization did not take place. Secretagogue activity was determined in mongrel female dogs of 10 kg weight with stainless steel gastric cannulas, and the peptides administered in saline i.v. with an infusion pump for a 1-hour period. Gastric juice was collected at 15-min intervals. To prevent dehydration, saline was infused during the entire experiment at a rate of 200 ml/h. The known acid and pepsin secretagogue, compound II named gastratet, has been studied in dogs in this laboratory [SACHATELLO *et al.*, 1971] and served as the standard for the comparison of the cyclic peptides.

In both cyclic peptides VI and XIII, a phenylalanine residue may be considered to acylate the α-amino group of tryptophan, and the α-amino group of tryptophan forms an amide with phenylalanine. Both peptides might, therefore, be expected to be secretagogues. However, both peptides were inactive at doses of 1, 3, 6 and 9 μg/kg/h, and did not alter the secretagogue activity of peptide II which is unequivocally active at 1 μg/kg/h. MORLEY [1968] has postulated that the aspartic acid residue is the active site of gastrin and the other amino acids are necessary for binding

to the cellular receptors. Had the cyclic peptides been bound even though they were themselves inactive as secretagogues, they would have been expected to inhibit the activity of peptide II.

The cyclic peptides differ from the linear analogs in that one of the hydrogen atoms of the amide groups of phenylalanine is substituted by a bulky group. However, MORLEY [1968] found 'significant activity' in the anilide and piperidine analogs in which, respectively, an aromatic 6-membered ring is substituted for one amide hydrogen, and a saturated 6-membered ring is substituted for both amide hydrogens. It thus appears unlikely that the formation of an amide of phenylalanine with the α-amino group of tryptophan is in itself inconsistent with biological activity.

The linear analog of peptide XIII, obtained by treating peptide IX with ammonia in methanol, was active as a pepsin and acid secretagogue, and the magnitude of the response suggested that both aspartic acid residues were functionally active sites [TRITSCH *et al.*, 1971]. This indicates that the dimeric structure is not inconsistent with biological activity *per se,* but that the restrictions imposed upon the three-dimensional conformation by the cyclic structure prevent the manifestation of hormone activity as well as binding to the cellular receptors.

It is concluded that the gastrin receptors on the chief and parietal cell membranes require a conformation of the hormone which the large rings of the cyclic peptides were unable to assume. Since rings of this size permit considerable numbers of three-dimensional conformations to be assumed, it appears that the gastrin receptors require not only the 4 amino acids in proper sequence, but a rather specific spatial arrangement as well.

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TI Cyclic peptides manufacture with Flexibacter
IN Teramura, Kyoko; Yasumuro, Kenichi; Suzuki, Yasuto; Shibazaki, Mitsuji; Abe, Kenji; Imai, Yoshimitsu; Suzuki, Kenichi
PA Yamanouchi Pharma Co., Ltd., Japan
SO Jpn. Kokai Tokkyo Koho, 14 pp.
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PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 06172385	A2	19940621	JP 1992-351725	19921208

OS MARPAT 122:131132
AB Cyclic peptides (I, R1 = benzylcarbonyl, isovaleryl; R2 and R3 are OH individually or together as carbonyl) and II are manufd. by culturing Flexibacter sp. I and II are inhibitors for esterase of leukocytes and are useful for treatment of lung diseases such as ARDS.

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